

## Histological Effects of Oral Administration of Artesunate on the Liver in Wistar Rats

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**Abstract:** This experiment was designed to study the histological effects of oral administration of normal and double normal doses of artesunate on the histology of the liver in wistar rats. The rats were divided into three groups (A, B and C) of five rats each. A and B served as the treatment groups, while C served as the control group. Group A rats were given 4 mg/kg b.w of artesunate daily for 3 days followed by 2 mg/kg b.w daily for next for 4 days. Group B rats were given 8 mg/kg<sup>2</sup> b.w of artesunate daily for 3 days followed by 4 mg/kg b.w daily for next 4 days, while group C rats were given only distilled water. The rats were fed with grower's mash purchased from Edo feeds and Flour Mill Ltd, Ewu, Edo state and were given water *ad libitum*. On day eight of the experiment, the rats were weighed and sacrificed by cervical dislocation. The livers were carefully dissected out and quickly fixed in 10% formal saline for histological studies. The histological findings after H and E method showed sinusoidal congestion with cytoplasmic vacuolation (hepatocyte oedema) and mild inflammation of the portal tracts. Our study suggests that artesunate at normal dose has a toxic effect on the liver cells and could be a potential hepatotoxic drug. It is therefore recommended that further studies aimed at corroborating these observations be carried out and self-medication with artesunate should be discouraged.

**Key words:** Antimalarial, artesunate, liver histology, toxicity

### INTRODUCTION

Malaria is a leading cause of mortality and morbidity in developing areas of the world, and remains a major public health problem in endemic regions (Bremner *et al.*, 2004). Resistance to available drugs is increasing, creating a need for new drugs that are well tolerated and simple to use. In the face of this ominous situation, artemisinin and its derivatives (artesunate, artemether, arteether, and dihydroartemisinin) have given renewed hope for combating resistant malaria (Hein, 1994; Harinasuta and Karbwang, 1994). These drugs have gained considerable prominence in the chemotherapy of both uncomplicated and severe falciparum malaria by demonstrating high activity against multidrug-resistant falciparum strains with low toxicity profiles (Chanthap *et al.*, 2005).

Artesunate is a drug used to treat malaria, especially chloroquine resistant malaria in Nigeria. It is a semi-synthetic derivative of artemisinin, the active compound of the Chinese herb *Artemisia annua*, which consists of the sodium succinyl salt of dehydroartemisinin

(Ittarat *et al.*, 1999). Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides; highly effective against multi-drug resistant strains of *Plasmodium falciparum* hence its increasingly wide usage for the treatment and management of malaria (Van Aghtmael *et al.*, 1999). It is used in combination therapy and is effective in cases of uncomplicated *P. falciparum*. Several studies on artesunate showed evidence of toxicity on the brain stem (Nontprasert *et al.*, 1998; Genovese, 2000; Nontprasert *et al.*, 2002), superior colliculus (Eweka and Adjene, 2008a), stomach (Eweka and Adjene, 2008b), and testis (Izunya *et al.*, 2010).

The liver is the largest solid organ in the body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver and is therefore susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the liver.

To my knowledge, there were no reports regarding the effects of artesunate on the histology of the liver. In view of this, the present study was carried out to

investigate and corroborate the previous work done on the biochemical toxicity of artesunate on the liver (Ngokere *et al.*, 2004; Nwanjo and Oze, 2007), by studying the effect of this antimalaria on the histology of the liver in wistar rats.

## MATERIALS AND METHODS

**Location and duration of study:** This study was conducted at the Histology Laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The preliminary studies, animal acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of one month (January, 2010). However, the actual administration of the drug to the test animals lasted for one week (15th, January to 21st, January 2010).

**Animals:** Fifteen adult wistar rats weighing between 100-150 g were used for this experiment. They were obtained and maintained in the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. They were divided into three groups A, B, and C of five rats each. Groups A and B were the treatment groups, while Group C served as the control. They were kept in each group per cage and fed with grower's mash produced by Bendel Feeds and Flour Mills Limited, Ewu, Nigeria. Water was given *ad libitum*. They were allowed to acclimatize for one week before commencement of the study. Ethical approval was sought and received from the Department of Anatomy, College of Medicine, Ambrose Alli University, Ekpoma, Edo State on the need to observe completely the rules guiding the employment of rats for scientific studies.

**Drug administration:** The artesunate tablets used for this experiment were manufactured by Mekophar Chemical Pharmaceutical Joint-Stock Company, Ho Chi Minh City, Vietnam and purchased from Irrua Specialist Hospital, Irrua, Edo State. The drug solution was made with distilled water (1 mg/mL) and administered to the animals by orogastric tube for a period of seven days. The dosage of artesunate was as per WHO recommendation of 4 mg/Kg body weight daily for 3 days followed by 2 mg/Kg body weight daily for the remaining 4 days. All the animals were weighed before the experiment. The drugs were administered to the groups as follows:

**Group A:** 4 mg/Kg body weight of artesunate daily for 3 days followed by 2 mg/Kg body weight daily for the remaining 4 days.

**Group B:** 8 mg/Kg body weight of artesunate daily for 3 days followed by 4 mg/Kg body weight daily for the remaining 4 days.

**Group C (Control) distilled water:** The graded daily doses gave us the opportunity of studying the effect of the normal and higher doses of the drug.

The animals were sacrificed by cervical dislocation 24 h after the last dose on the 8th day of the respective treatment and the livers were harvested.

**Histological study:** For light microscopic examination, liver tissues from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5 µm thickness and stained with haematoxylin and eosin (Drury *et al.*, 1967). The photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

## RESULTS AND DISCUSSION

Histological analyses of the liver of rats in Group C showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins.

Histological analyses of the liver of rats in Group A showed mild cytoplasmic vacuolation (hepatocytes oedema) and sinusoidal congestion (Plate 1).

Histological analyses of the liver of rats in Group B showed severe sinusoidal congestion with severe cytoplasmic vacuolation (severe hepatocyte oedema) and mild inflammation of the portal tracts (Plate 2).

Histological results suggest toxicity of the liver cells of the wistar rats upon artesunate administration. This was shown by the sinusoidal congestion with cytoplasmic vacuolation (hepatocyte oedema) and mild inflammation of the portal tracts. These changes were apparently dose dependent.

The findings in this study agree with the work of Nwanjo and Oze (2007) in which artesunate

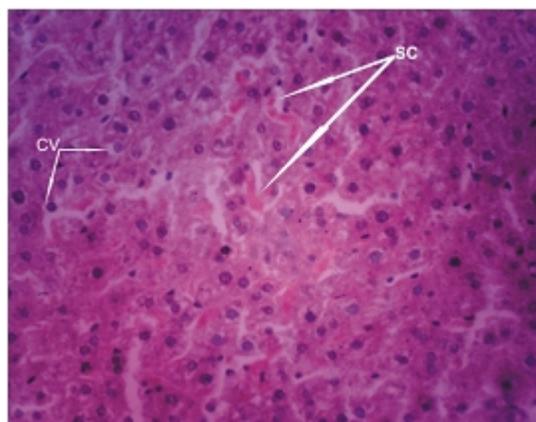


Plate 1: (Group A) Treatment section of the liver that received 4 mg/kg for 3 days and thereafter 2 mg/kg for 4 days of artesunate (Mag. X400), showing mild Cytoplasmic Vacuolation (CV) and Sinusoidal Congestion (SC)

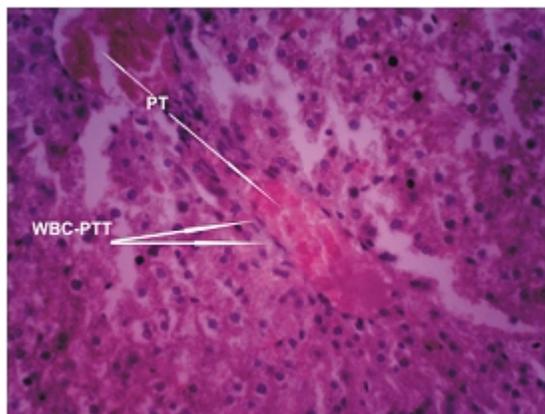


Plate 2: (Group B) Treatment section of the liver that received 8 mg/kg for 3 days and thereafter 4 mg/kg for 4 days of artesunate (Mag. X400), showing severe Cytoplasmic Vacuolation (CV) and Portal Triaditis (PTT). WBC = White blood cells, PT = Portal Tract

administration was found to be hepatotoxic in guinea pigs. They also agree with the work of Ngokere *et al.* (2004), in which artesunate administration caused significant increase in the liver marker enzymes in rabbit.

Degenerative changes have been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death (Cohen, 1993; Vaux *et al.*, 1994). These two types differ morphologically and biochemically (Bose and Sinha, 1994). Apoptosis is a non-inflammatory response to tissue damage characterized by a series of morphological and biochemical changes (Sakkas *et al.*, 1999; Sinha and Swerdloff, 1999; Shen *et al.*, 2002; Grunewald *et al.*, 2005). Apoptosis can be triggered in two principal ways: by toxic chemicals or injury leading to damage of DNA or of other important cellular targets, and activation or inactivation of receptors by growth-regulating signal factors in the organism (Schulte-Hermann *et al.*, 1999).

Initiation of apoptosis can result from multiple stimuli, including heat, toxins, ROS, growth factor withdrawal, cytokines such as transforming growth factor-beta, loss of matrix attachment, glucocorticoid, nitric oxide, and radiation (Thompson, 1995; Pollman *et al.*, 1996). These stimuli work in conjunction with other intrinsic factors that determine the cell's potential to undergo apoptosis (McConkey and Orrenius, 1991). However, high levels of ROS disrupt the inner and outer mitochondrial membranes, inducing the release of the cytochrome-C protein and activating the caspase cascade which ultimately results in the fragmentation of a cell's DNA (Wyllie, 1980; Green, 1998; Makker *et al.*, 2009).

Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects (Farber *et al.*, 1981). The process of cellular necrosis

involves disruption of the membranes structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in Programmed Cell Death (PCD), but by an abrupt environmental perturbation and departure from the normal physiological conditions (Martins *et al.*, 1978).

Generally, artesunate exerts its anti-malarial activity by the generation of Reactive Oxygen Species (ROS) from its endoperoxide bond (Maggs *et al.*, 1988) leading to lipid peroxidation (Robert *et al.*, 2001). The accumulation of lipid peroxides is toxic to the membrane structure, leading to a change in permeability and to disintegration of cellular organelles (Muller and Ohnesorge, 1982).

ROS generation is a normal component of oxidative phosphorylation and plays a role in normal redox control of physiological signaling pathways (Murdoch *et al.*, 2006; Sawyer *et al.*, 2002; Giordano, 2005). However, excessive ROS generation triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death (Murdoch *et al.*, 2006; Sawyer *et al.*, 2002; Giordano, 2005). Moreover, there are also reports that cadmium toxicity in liver may be mediated by the production of reactive oxygen species known to induce necrosis in various rat organs (Razinger *et al.*, 2008; Hsu *et al.*, 2007), lipid peroxidation (Borges *et al.*, 2008) and a decrease in antioxidant enzymes (El-Sharaky *et al.*, 2007).

ROS are small, oxygen-based molecules that are highly reactive because of unpaired electrons (Papa and Skulachev, 1997). The most prominent ROS are the superoxide anion ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl ion ( $OH^{\bullet}$ ) (Turner and Lysiak, 2008). Cells also have intrinsic antioxidant systems that counter ROS accumulation. These include enzymes such as catalase, glutathione peroxidases, and superoxide dismutase, and nonenzymatic antioxidants, such as vitamins E, C, beta carotene, ubiquinone, lipotic acid, and urate (Giordano, 2005; Nordberg and Arner, 2001). Nevertheless, under several situations, the rate of generation of ROS exceeds that of their removal and oxidative stress occurs (Giordano, 2005; Di-Giulio *et al.*, 1995; Halliwell and Gutteridge, 2000; Livingstone, 2001). However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon *et al.*, 1991). However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart (Lelli *et al.*, 1998; Lee *et al.*, 1999).

In this study, artesunate may have acted indirectly through generation of high levels of ROS or directly as toxin to the cells of the liver, affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis. In cellular necrosis, the rate

of progression depends on the severity of the environmental insults. The greater the severity of the insults the more rapid the progression of neuronal injury (Ito *et al.*, 2003). The principle holds true for toxicological insult to the brain and other organs (Martins *et al.*, 1978). Thus, it may be inferred from this result that normal and double normal dose of artesunate resulted in toxic effects on the liver.

### CONCLUSION

Our study suggests that artesunate at normal dose is hepatotoxic. Thus, there is a need to determine if these observations in wistar rats may be applicable to humans and in this regard, one can suggest that artesunate at normal dose could be a potential hepatotoxic agent. It is therefore recommended that further studies aimed at corroborating these observations be carried out and self-medication involving artesunate should be discouraged.

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